



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/68 // C07K 15/06		A1	(11) International Publication Number: WO 91/15773 (43) International Publication Date: 17 October 1991 (17.10.91)
(21) International Application Number:	PCT/GB91/00545	(74) Agent:	BANNERMAN, David, Gardner; Withers & Rogers, 4 Dyer's Buildings, Holborn, London EC1N 2JT (GB).
(22) International Filing Date:	8 April 1991 (08.04.91)	(81) Designated States:	AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), SU, US.
(30) Priority data: 9007922.9	7 April 1990 (07.04.90)	GB	(71) Applicant (<i>for all designated States except US</i>): OXFORD VIROLOGY PLC [GB/GB]; 10 Storey's Gate, Westminster, London SW1P 3AY (GB).
(72) Inventors ; and (75) Inventors/Applicants (<i>for US only</i>) : HAJIMOHAMMADREZA, Iradj [IR/GB]; Department of Neuroscience, Institute of Psychiatry, London SE5 8F (GB). BRAMMER, Michael [GB/GB]; 29 Rodenhurst Road, London SW4 8AE (GB).			Published <i>With international search report.</i>
(54) Title: A DIAGNOSTIC TEST METHOD			
(57) Abstract			
<p>The invention provides a method for diagnosing Alzheimer's Disease comprising detecting the amount of one or more polypeptides in a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease sufferer and, if present, comparing the amount thereof with the amount of the polypeptide or polypeptides present in a corresponding sample taken from disease-free individuals. Significantly lowered amounts of said polypeptide or polypeptides in the sample, when compared to the amount in samples from disease-free individuals, being indicative of Alzheimer's Disease.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark				

A DIAGNOSTIC TEST METHOD

This invention relates to a method of diagnosis for Alzheimer's Disease.

Alzheimer's Disease is a degenerative brain disorder producing a range of symptoms including dementia. Elevated aluminium levels, virus-like agents and predisposing genetic factors have all been put forward as causal agents. However, the absence of a specific diagnostic test for Alzheimer's Disease has greatly hampered further research and the development of therapeutic agents for the treatment of afflicted individuals.

This invention exploits the finding that certain polypeptides normally found in blood platelets are present in significantly lowered amounts or are absent from the platelets of Alzheimer's Disease sufferers.

According to one aspect, the invention provides a method of diagnosing Alzheimer's Disease comprising detecting the presence of one or more polypeptides in a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease sufferer, and if such a polypeptide or polypeptides are present, comparing the amount thereof with the amount present in the platelets of disease-free individuals.

In a further aspect, the invention provides a method of diagnosing Alzheimer's Disease comprising separating the components of a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease sufferer, detecting the presence of the separated polypeptides and comparing the amount thereof with the amount of the corresponding polypeptide observed in a corresponding sample taken from a disease-free individual.

As will be apparent to the skilled worker, many separation techniques can be employed to separate particular polypeptides on the basis of such characteristics as size, solubility, charge and specific binding affinity, using methods such as dialysis through a semi-permeable membrane; gel-filtration chromatography; ion-exchange chromatography and affinity chromatography (see Stryer L. Biochemistry 2nd Edition, [1981] Pg. 18 to 20, W.H. Freeman and Co.).

The separation of the components may, for example, be carried out by chromatography or by gel electrophoresis such as polyacrylamide gel electrophoresis (PAGE). Preferably two dimensional PAGE is used to separate the polypeptide components in the samples.

Detection techniques such as immunoassay and colourimetry may be employed in the above methods to determine the presence and/or amount of particular polypeptides in a test sample.

In another aspect the invention provides a diagnostic test method for Alzheimer's disease comprising

detecting or determining the level of one or more particular polypeptides in a solubilised blood platelet polypeptide sample taken from a test subject, wherein the or each particular polypeptide is one which, if the components of the said sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2.

The invention further provides a method of diagnosing Alzheimer's Disease comprising separating the components of a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease sufferer by two dimensional PAGE, detecting the presence of the separated polypeptides by staining the gel, and comparing the degree of band staining, in one or more regions selected from the 100 Mwt region at an isoelectric point of approximately 5.4, the 50 kDa Mwt region at an isoelectric point of approximately 5.5, and the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2, with the degree of staining in the corresponding region or regions observed in a sample taken from a disease-free individual.

Advantageously, the gels are run in pairs, that is one sample from a suspected sufferer with one from a disease-free individual to account for any variability in running conditions.

Advantageously, the samples taken from suspected sufferers and disease-free individuals are matched as closely as possible for age and sex so that samples are more strictly comparable.

The presence of the separated polypeptide bands on the gel may be detected using a polypeptide stain such as "Coomassie blue", or by using immunoassay techniques.

Preferably, the or each particular polypeptide is extracted from the sample or gel using conventional techniques and is used to immunise a mammal such as a rabbit in order to raise antisera specific for said polypeptide. Such antisera can be used as an immunoassay reagent in further methods of the invention.

In a further aspect the invention provides a method for separating from a solubilised blood platelet sample a polypeptide which, if the components of the sample are subjected to two-dimensional polyacrylamide gel electrophoresis, is present in the 110 kDa Mwt

region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2 which method comprises:

- (i) applying said sample to an affinity chromatography column including antibodies specific for said polypeptide, which antibodies are bound to a solid support;
- (ii) washing the support to remove unbound components of the sample to which the antibodies do not bind specifically;
- (iii) eluting said polypeptide from the support so that it is obtained in substantially purified form.

Preferably the solid support to which said antibodies bind comprises plastic beads.

The invention also provides said polypeptide in an isolated and substantially purified form, which polypeptide is present in a reduced concentration in a solubilised blood platelet sample from a sufferer from Alzheimer's disease and which, if the components of such a sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point

of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2.

In a still further aspect the invention provides an immunoassay method for determining the amount of a particular polypeptide as described previously which method comprises

- (i) coating a solid support surface with antibodies specific for the particular polypeptide;
- (ii) washing the support to remove unbound antibodies;
- (iii) applying a solubilised blood platelet polypeptide sample from a test sample;
- (iv) washing the support to remove unbound components of the sample;
- (v) applying labelled antibodies specific for the particular polypeptide;
- (vi) washing the support to remove any unbound labelled antibodies;
- (vii) determining the amount of labelled antibody present, which amount is proportional to the amount of the particular polypeptide present in the sample.

Preferably the labelled antibodies are labelled with enzymes (such as peroxidase and phosphatase) which

leave a coloured deposit on reaction with substrate.

The coloured deposit being measurable using colourmetric techniques.

In another aspect the invention provides an immunoassay kit for diagnosing Alzheimer's Disease in a solubilised blood platelet polypeptide sample from a test subject comprising

- (i) an antibody preparation containing antibodies specific for a particular polypeptide as defined previously;
- (ii) An antibody preparation containing labelled antibodies specific for the said polypeptide for determining the amount of said polypeptide in the sample from the test subject; and
- (iii) a comparative blood platelet polypeptide sample from an Alzheimer's disease-free individual to allow comparison of the amount of said polypeptide therein with the amount present in the sample from the test subject.

Preferably the above kit further comprises a multiwell plastic plate for using the kit according to the methods of the invention. Advantageously the sample from the test subject is assayed alongside the comparative sample from the disease-free individual so

that the amount of the particular polypeptide in the respective samples can be readily compared.

As will be apparent to those skilled in the art examples of the label of the labelled antibodies include an enzyme as mentioned previously, a fluorescent dye which can be seen using UV microscopy, or a radioactive substance which can be detected by deposition of silver grains from a photographic emulsion or by using counting equipment.

A preferred embodiment of the invention will now be described by way of example only, with reference to Fig. 1.

Fig. 1 shows a diagnostic test according to the invention.

A total of 13 2-D SDS PAGE were carried out to separate the components of solubilised platelet polypeptide samples.

Eight Alzheimer's Disease (ALZ) patients and 5 disease-free control (CON) individuals were tested. These were matched as closely as possible for age (63 + 4 years for ALZ group and 62 + 5 years for CON group) and sex (4 males/4 females for ALZ group and 3 males/2

females for CON group).

Gels were run in pairs, that is, one ALZ with one CON, to account for the variability in running conditions.

Coomassie blue staining was used to detect the presence of the separated polypeptide bands.

In 5 out of 8 SDS-gels from the ALZ group, there was a consistent decrease in the degree of Coomassie blue staining of a spot around the 110 kDa Mwt region with an isoelectric point of approximately 5.4, as shown in Fig. 1, B=ALZ, marked 1. However, all 5 SDS-gels from the control group showed a much stronger staining as shown in Fig. 1, A=CON, marked 1.

Further differential staining was observed in the spot around the 50 kDa Mwt region with an isoelectric point of approximately 5.5, marked 2 in Fig. 1 and the spot marked 3 around the 40 kDa Mwt region with an isoelectric point in the range 5.6 to 6.2.

Thus, the differential staining of certain blood platelet polypeptide bands between Alzheimer's Disease patients and controls provides a specific marker or markers for the diagnosis of the disease in suspected sufferers.

SUBSTITUTE SHEET

CLAIMS

1. A diagnostic test method for Alzheimer's Disease characterised in that at least one polypeptide of a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease suffer is detected and, if present, the amount thereof is compared with the amount of the same polypeptide in a corresponding sample taken from a disease-free individual.
2. A diagnostic test method for Alzheimer's disease comprising detecting or determining the level of one or more particular polypeptides in a solubilised blood platelet polypeptide sample taken from a test subject, wherein the or each particular polypeptide is one which, if the components of the said sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2.
3. A method as claimed in claim 1 wherein the components of the solubilised blood platelet polypeptide sample are separated prior to the

SUBSTITUTE SHEET

detection of the polypeptide.

4. A method as claimed in claim 3 wherein the separation of the components is achieved by two dimensional polyacrylamide gel electrophoresis.

5. A method as claimed in claim 2 wherein the presence of the separated polypeptides in the gel is detected by staining the gel and comparing the degree of band staining in one or more regions selected from the 110 kDa Mwt region at an isoelectric point of 5.4, the 50 kDa Mwt region at an isoelectric point of 5.5 and the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2, with the degree of staining in the corresponding region or regions in a corresponding sample taken from a disease-free individual.

6. A method for separating from a solubilised blood platelet sample a polypeptide which, if the components of the sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2 which method comprises:

- (i) applying said sample to an affinity chromatography column including antibodies specific for said polypeptide, which antibodies are bound to a solid support;
- (ii) washing the support to remove unbound components of the sample to which the antibodies do not bind specifically;
- (iii) eluting said polypeptide from the support so that it is obtained in substantially purified form.

7. A polypeptide in isolated and purified form, which polypeptide is present in a reduced concentration in a solubilised blood platelet sample from a sufferer from Alzheimer's disease and which, if the components of such a sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2.

8. A method as claimed in claim 1 wherein the particular polypeptide or polypeptides are detected by immunoassay.

9. An immunoassay method for determining the amount

SUBSTITUTE SHEET

of a particular polypeptide as claimed in claim 1 or 2 comprises:

- (i) coating a solid support surface with antibodies specific for the particular polypeptide;
- (ii) washing the support to remove unbound antibodies;
- (iii) applying a solubilised blood platelet polypeptide sample from a test sample;
- (iv) washing the support to remove unbound components of the sample;
- (v) applying labelled antibodies specific for the particular polypeptide;
- (vi) washing the support to remove any unbound labelled antibodies;
- (vii) determining the amount of labelled antibody present, which amount is proportional to the amount of the particular polypeptide present in the sample.

10. An immunoassay kit for diagnosing Alzheimer's disease in a solubilised blood platelet polypeptide sample from a test subject comprises:

- (i) an antibody preparation containing antibodies specific for a particular polypeptide as defined in claim 1 or 2;
- (ii) An antibody preparation containing labelled

antibodies specific for the said polypeptide
for determining the amount of said
polypeptide in the sample from the test
subject; and

- (iii) a comparative blood platelet polypeptide
sample from an Alzheimer's disease-free
individual to allow comparison of the amount
of said polypeptide therein with the amount
present in the sample from the test subject.

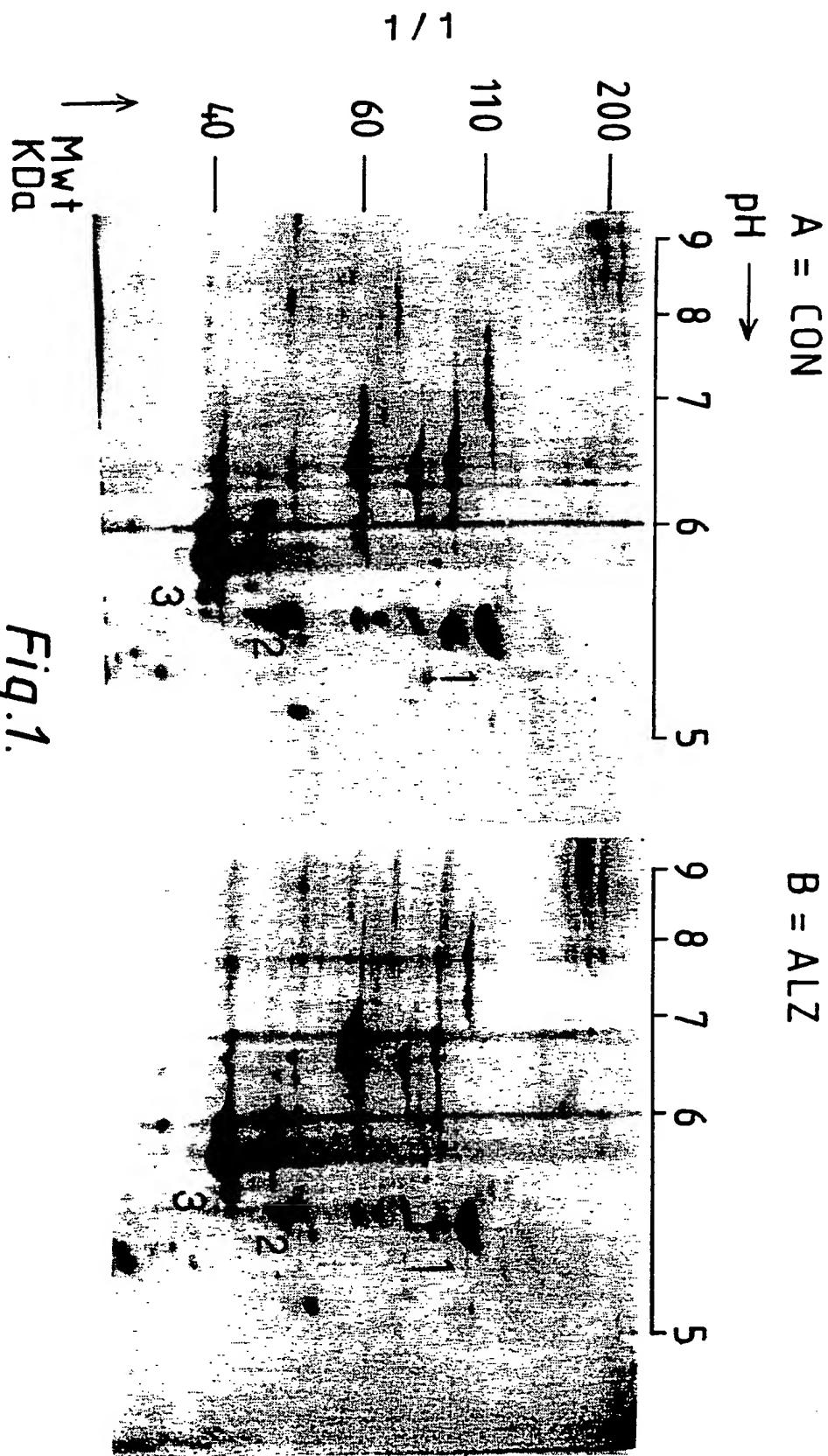


Fig.1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00545

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: G 01 N 33/68 // C 07 K 15/06

II. FIELDS SEARCHED

Minimum Documentation Searched ?	
Classification System	Classification Symbols
IPC ⁵	G 01 N, G 07 K
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *	

III. DOCUMENTS CONSIDERED TO BE RELEVANT*

Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y,P	Science, volume 248, no. 4956, May 1990, W.E. Van Nostrand et al.: "Protease nexin-II (amyloid β -protein precursor): A platelet α -granule protein", pages 745-748 see the whole document .	1,3,4,8
A	--	2,5-7,9-10
Y	US, A, 4666829 (G.G. GLENNER et al.) 19 May 1987 see the whole document	1,3,4,8
A	--	
A	Biotechnology, volume 7, February 1989, C.R. Abraham et al.: "Alzheimer's disease: Recent advances in understand- ing the brain amyloid deposits", pages 147-152 see pages 147-148; table 1	1
	--	
	. / .	

* Special categories of cited documents: ¹⁰

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19th June 1991	31 JUL 1991
International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer <i>[Signature]</i> MISS T. TAZELAAR

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO, A, 8907657 (THE CHILDREN'S MEDICAL CENTER CORP.) 24 August 1989 see abstract; pages 4-5, pages 15-16; claims 10-20 --	1-10
P,A	The Journal of Biological Chemistry, volume 265, no. 26, 15 September 1990, The American Society for Biochemistry and Molecular Biology, Inc., (US), A.I. Bush et al.: "The amyloid precursor protein of Alzheimer's disease is released by human platelets", pages 15977-15983 see the whole document --	1-10
P,A	Science, volume 248, no. 4959, 1 June 1990 R.P. Smith et al.: "Platelet coagulation factor XIa-inhibitor, a form of Alzheimer myloid precursor protein", see the whole document -----	1-10

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. GB 9100545
SA 46702**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 22/07/91. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4666829	19-05-87	CA-A-	1268131	24-04-90
WO-A- 8907657	24-08-89	AU-A-	3204689	06-09-89

**HPS Trailer Page
for
WEST**

**UserID: phuynh
Printer: cm1_8e12_gblaptr**

Summary

Document	Pages	Printed	Missed	Copies
WO009115773	20	20	0	1
Total (1)	20	20	0	-